Chapter 2

Welfare Indicators Correlated with Resistance to Disease: The Romanian Experience

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Additional information is available at the end of the chapter

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Abstract

The chapter aims to reveal the importance of indicators in defining equine welfare and their relationship with the immune system and subsequent resistance to diseases. The sharp economic changes after the World War II changed the role played by horses in the society. The improvement of modern breeds, their involvement in different equestrian activities, the development of sports horse shows and industry, the increase of the economic value of the individuals on the basis of their performances, as well as the emergence of modern "natural" training theories implicitly led, in developed countries, to a change of public attitude toward this animal. According to World Organization for Animal Health (OIE) principles, animal welfare and animal health are closely linked. The correlated use of the welfare assessment by direct indicators with the investigation of the immune status proved to be a powerful tool in the interdisciplinary research on how the horses cope with different management conditions and the health outcome of different raising technologies. The dimensions of this approach have not been exhausted by far in this study which is merely a first step taken in Romania to a better understanding on protection and use of these animals.

Keywords: horses, welfare indicators, indicator ranking, seasonal variability, raising system

1. Introduction

The World Organization for Animal Health (OIE) defines animal welfare as a multifaceted public policy issue that includes scientific, ethical, economic and political dimensions Belton [1]. Concerns about animal welfare and the knowledge available in this area nowadays represent the sum-up of the results that emerged and accumulated from numerous studies conducted over the years. The principles on animal welfare underlying OIE very well synthesize

the level where this area finds itself currently as follows: (1) animal welfare and animal health are closely linked; (2) the animal's five freedoms lie at the basis of well-being; (3) animal welfare standards are scientifically well-founded; (4) ensuring animal welfare safeguards the well-being of mankind; (5) the use of animals must be carried out with ethical responsibility in order to ensure their well-being; (6) a high level of animal welfare would increase animal productivity and would provide economic benefits; (7) equivalent results based on performance criteria are the basis for comparing animal welfare standards [1].

Although the expansion of scientific research and the number of experimental researches in all sectors where horses are used is very high, it seems that so far no systematization and/or synthesis of the results have been achieved to provide neither practical assessment tools nor methods for influencing equine status. There is a high inconsistency of the nature and content of legal documents referring to horses, of the descriptions of activity fields in which horses are used, and of the ways these animals are perceived by owners/caretakers in different countries of the world.

During the twentieth century, the massive reduction in military and agricultural uses of horses in the developed countries occurred simultaneously with the increase in the numbers of animals used for sports, competitions and leisure [2]. The improvement of modern breeds, their involvement in different equestrian activities, the development of sports horse shows and industry, the increase of the economic value of the individuals on the basis of their performances, as well as the emergence of modern "natural" training theories implicitly led, in developed countries, to a change of public attitude toward these animals. From the economic point of view, there are countries that receive considerable income from equestrian activities. According to the International Equestrian Federation (EIF), Europe's equine industry is a growing sector, with the number of riders rising annually by about 5% [3].

Equine, as any other mammalian well-being status, is connected with resistance to disease. Due to their versatility, horses, when compared to other farmed species, are even more exposed to various types and levels of stress, thus showing a high variability of immune responses and subsequent susceptibility to microbial infections. Technologies include immunological prevention of microbial diseases by vaccination, but welfare indicators are also very important in measuring the outcome of these operations.

2. Welfare indicators in Romanian horses

2.1. The welfare assessment of the studied horses: working protocol

Horses under two different raising systems, private and state stud, were subjected to the evaluation [4]. The animals were assessed in two different seasons: privately owned working horses (171 animals in the winter and 168 in the summer) and breeding horses (breeding stallions: 62 heads in the winter and 66 in the summer; broodmares and young horses: 137 animals in the winter and 146 in the summer; reproduction stallions: 14 heads in the winter and the same 14 during the summer). The keeping and housing conditions were highly

variable in the privately owned horses, mostly in barns along with other domestic animals of the household. The breeding horses as the property of a state stud were kept under standard conditions regulated by the national law.

This chapter describes the methodology of development and initial testing of the horse welfare assessment protocol, than presents its final version [5–9]. The protocol was constructed using 30 indicators (selected based on their relevance in a previous field-testing). Out of all the indicators, only four were indirect, resource based measures. The indicators were grouped based on the five freedoms of animal welfare, each of these investigating specific aspects of one of the freedoms. Being a practical tool, the protocol contains the detailed methodology for the assessment of each indicator (specifying the source in case of adaptations from other authors), the possible scores and their assignment methods, providing also exemplifying pictures for the majority of the measures. A specific feature of this protocol was the assignment of minimal scores for the negative variants of the indicators and of the maximal scores where the animal showed good welfare status. In addition to the evaluation based on the assessment protocol, supplementary data were recorded, based on the responses of the owners to a questionnaire.

The individual welfare score was calculated by summing up the scores for each assessed indicator; the classification of the horses in qualitative welfare categories was based on the use of a grid with numerical limits. All the animals included in the study were assessed using this protocol.

In order to evaluate the freedom from hunger and thirst, the body condition score was employed and data were recorded regarding the frequency and ways of watering for each horse (owners' questionnaires based answers). For the investigation of the freedom from discomfort, scores were assigned depending on the fecal soiling of the hindquarters and of the lateroventral abdomen and for the presence or absence of skin lesions on the hip point. During the assessment of freedom from pain, injury and disease an inspection was made concerning the quality of the horses' hair coat, mane and tail, presence of skin lesions (on the body, on the lower part of the legs, on lip corners and in the harness contact points), swellings of tendons/joints, hoof horn quality, the shape of the hoof, the quality of the sole, the length of the hooves, the aspect of the shoes and shoeing quality, the presence or absence of hampered respiration, secretions from the nostrils or eyes, coughing, diarrhea; also the presence of sight was tested and the owners were asked about checking of the horses' teeth. For this part of the assessment, the gait of the horses was also inspected. The freedom to express normal behaviors consisted of asking the owners about the social company (of their conspecifics) of the horses and their access to free exercise. For the investigation of the freedom from fear and distress, the general alertness of the animals was observed and three behavioral tests were employed to explore the quality of the human-horse relation.

The results were centralized and processed for the analysis of the welfare indicators. The prevalence of each possible variant for each indicator was calculated; these were afterward compared among categories and seasons. To offer an overview of the most important results, only those indicating welfare problems of the assessed horses were presented here (**Tables 1–5**).

Indicators	% in each season and category								P
	WHw	WHs	BSw	BSs	BMw	BMs	RSw	RSs	
I. FREEDOM FROM HUNGER AND THIRST									
Body Condition Score (BCS)									
BCS unacceptable	23.98^{a}	18.45^{ab}	1.61^{c}	0^{c}	15.34^{abc}	8.22^{bc}	0^{abc}	0^{abc}	P<0.05
Watering Pattern and frequency									
1.2 times/day	5.26	4.76	0	0	0	0	0	0	P>0.05

Table 1. The prevalence of welfare problems identified in the horses in different keeping conditions and seasons, within the investigation of the freedom from hunger and thirst. WHw = working horses, in winter; WHs = working horses, in summer; BSw = breeding stallions, in winter; BSw = breeding stallions, in summer; BMw = broodmares and young horses, in winter; BMs = broodmares and young horses, in summer; RSw = reproduction stallions, in winter; RSs = reproduction stallions, in summer; ns = difference not statistically significant (P > 0.05); P = significance of difference between categories and seasons; abc Values in a row with no common superscript are significantly different (P < 0.05).

2.2. Results and discussion

The prevalence of unacceptable body condition scores in horses was lower than that indicated in reports of the studies assessing working horses in the developing countries. Except for the working horse category evaluated during winter, the majority (60.12–100%) of the animals had good body condition. Almost a quarter of the working horses were found in unacceptable body condition in the winter (**Table 1**) and such cases were also observed in other horse categories, at a lower extent. The inadequately low watering frequency (once or twice per day) was recorded exclusively in the working horses (**Table 1**). Nevertheless, from all the categories, only the reproduction stallions had unlimited access to drinking water. Together with other factors (insufficient feeding in the first place), the reduced water consumption lead to body weight losses in horses, by limiting the feed ingestion and lowering the efficiency of the nutrient usage.

The assessment of barn hygiene based on the body hygiene of the animals is frequently used in dairy cows [10–13], but not in horses, since it is considered that the owners clean their horses whenever they leave the stable. The high prevalence of horses having their hindquarters and lateroventral abdomen soiled by dejections proved that this indicator was fit to the conditions of the present study, but also demonstrated the ignorance of the owners regarding the body hygiene of their animals. The prevalence of horses with dirty rumps showed significant differences among the studied categories, but also between the seasons (**Table 2**). The horses kept on pasture or housed in stables with a decent amount of bedding and mechani-

Indicators	% in each season and category								P
	WHw	WHs	BSw	BSs	BMw	BMs	RSw	RSs	
II. FREEDOM FROM DISCOMFORT									
Dirty hindquarters and lateroventral abdomen									
Dirty	54.97^{a}	45.24^{a}	0^{bc}	0^{bc}	16.79^{b}	0^{c}	0^{bc}	0^{bc}	P<0.05
Lesions on the external points of the ileum									
Wounds	15.79^{a}	11.4^{a}	0^{b}	0^{b}	0^{b}	0^{b}	0^{ab}	0^{ab}	P<0.05

Table 2. The prevalence of welfare problems identified in the horses in different management conditions and seasons, within the investigation of the freedom from discomfort. WHw = working horses, in winter; WHs = working horses, in summer; BSw = breeding stallions, in winter; BSw = breeding stallions, in summer; BMw = broodmares and young horses, in winter; BMs = broodmares and young horses, in summer; RSw = reproduction stallions, in winter; RSs = reproduction stallions, in summer; ns = difference not statistically significant (P > 0.05); P = significance of difference between categories and seasons; abc Values in a row with no common superscript are significantly different (P < 0.05).

cal cleaning in place did not show fecal soiling of their bodies, in spite of not being groomed daily. These results confirmed the importance of stable cleaning and bedding usage for the horses' comfort of rest [14].

Indicators	% in each season and category								P
	WHw	WHs	BSw	BSs	BMw	BMs	RSw	RSs	
III. FREEDOM FROM PAIN, INJURY AND DISEASES									
Hair coat quality									
Abnormal	30.4^{a}	27.98^{ab}	9.67^{bc}	16.67^{abc}	16.06^{bc}	10.27^{c}	0^{abc}	0^{abc}	P<0.05
Quality of the mane and tail									
Abnormal	20.47^{a}	20.84^{a}	11.29^{a}	7.58^{a}	16.06^{a}	6.85^{a}	35.71^{a}	21.43	P<0.001
Body lesions									
Superficial	29.82^{ab}	33.93^{a}	11.29^{c}	15.15^{bc}	10.22^{c}	7.53^{c}	7.14^{abc}	0^{abc}	P<0.05
Lesions on the lower parts of the legs									
Severe	6.44^{ab}	10.71^{a}	8.06^{ab}	6.06^{ab}	0.73^{b}	0^{b}	0^{ab}	0^{ab}	P<0.01
Lip corner lesions									
Present	22.23^{a}	24.4^{a}	9.68^{ab}	6.06^{b}	0^{b}	0^{b}	0^{ab}	0^{ab}	P<0.05
Harness contact point lesions									
Wounds	21.64^{a}	19.05^{a}	0^{b}	0^{b}	0^{b}	0^{b}	0^{ab}	0^{ab}	P<0.001
Swellings of tendons/joints									
Tendon and joint	17.54^{a}	21.43^{a}	9.68^{ab}	12.12^{ab}	0^{b}	0^{b}	0^{ab}	0^{ab}	P<0.001
Hoof horn quality									
Abnormal	40.94^{a}	39.91^{a}	27.42^{ab}	21.82^{ab}	5.11^{c}	0^{c}	0^{bc}	0^{bc}	P<0.05
Shape of the hoof									
Indicators	**% in each season and category**								**P**
	WHw	**WHs**	**BSw**	**BSs**	**BMw**	**BMs**	**RSw**	**RSs**	
Abnormal	33.33^{a}	25^{ab}	8.06^{bc}	6.06^{c}	10.95^{c}	7.53^{c}	0^{bc}	0^{bc}	P<0.05
Sole surface quality									
Abnormal	24.56^{a}	23.22^{a}	6.45^{b}	7.58^{b}	5.11^{b}	0^{b}	0^{ab}	0^{ab}	P<0.05
Too long/too short hooves									
Yes	52.63^{a}	52.38^{a}	32.26^{ab}	24.24^{b}	13.87^{b}	12.33^{b}	14.28^{ab}	21.43^{ab}	P<0.01
Improper shoes									
Yes	61.98^{b}	71.43^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{b}	P<0.001
Abnormal gait									
Abnormal gait	13.45^{a}	17.26^{a}	8.06^{ab}	9.05^{ab}	2.92^{b}	2.05^{b}	0^{ab}	0^{ab}	P<0.05
Dispnea (hampered respiration)									
Present	33.34^{a}	22.62^{a}	14.52^{ab}	15.15^{ab}	4.38^{b}	2.05^{b}	0^{b}	0	P<0.01
Coughing									
Present	19.3^{a}	13.1^{a}	6.45^{ab}	10.6^{ab}	0^{b}	0^{b}	0^{ab}	0^{ab}	P<0.01
Nasal secretions									
Present	17.54^{a}	11.31^{ab}	8.06^{ab}	0^{b}	3.65^{b}	2.05^{b}	0^{ab}	0^{ab}	P<0.001
Eye secretions									
Present	10.53^{a}	5.96^{ab}	6.45^{ab}	4.54^{ab}	1.46^{b}	0^{b}	0^{ab}	0^{ab}	P<0.01
Blindness									
Both eyes	1.75	4.17	4.84	0	0	0	0	0	P>0.05
Diarrhea									
Present	9.36^{ab}	10.71^{a}	0^{bc}	4.54^{abc}	0^{c}	0^{c}	0^{abc}	0^{abc}	P<0.05
Checking of teeth									
Never	88.31^{ab}	92.26^{a}	93.55^{a}	86.36^{ab}	93.43^{a}	92.46^{a}	71.43^{ab}	64.28^{b}	P<0.05

Table 3. The prevalence of welfare problems identified in the horses in different management conditions and seasons, within the investigation of the freedom from pain, injury and disease. WHw = working horses, in winter; WHs = working horses, in summer; BSw = breeding stallions, in winter; BSw = breeding stallions, in summer; BMw = broodmares and young horses, in winter; BMs = broodmares and young horses, in summer; RSw = reproduction stallions, in winter; RSs = reproduction stallions, in summer; ns = difference not statistically significant (P > 0.05); P = significance of difference between categories and seasons; abc Values in a row with no common superscript are significantly different (P < 0.05).

Indicators	% in each season and category								P
	WHw	WHs	BSw	BSs	BMw	BMs	RSw	RSs	
IV. FREEDOM TO EXPRESS NORMAL BEHAVIORS									
Social company									
None	26.31^{ab}	31.55^{a}	0^{c}	0^{c}	0^{c}	0^{c}	0^{bc}	0^{bc}	P<0.05
Access to free exercise									
None	88.89^{a}	60.12	100^{a}	100^{a}	0^{b}	0^{b}	0^{b}	0^{b}	P<0.001

Table 4. The prevalence of welfare problems identified in the horses in different keeping conditions and seasons, within the investigation of the freedom to express normal behavior. WHw = working horses, in winter; WHs = working horses, in summer; BSw = breeding stallions, in winter; Table 4 legend: BSw = breeding stallions, in summer; BMw = broodmares and young horses, in winter; BMs = broodmares and young horses, in summer; RSw = reproduction stallions, in winter; RSs = reproduction stallions, in summer; ns = difference not statistically significant (P > 0.05); P = significance of difference between categories and seasons; abc Values in a row with no common superscript are significantly different (P < 0.05).

The presence of the hip point (ileum) lesions was used as an indicator of the comfort around resting only in Romania. The preexistent individual conditions accelerating the development of decubital lesions on the bony parts of the animals' body include the loss of the natural padding in thin horses but also the precarious hygiene of the skin. Yet, it seems that the main factor triggering these injuries is the inappropriate quality of the resting surface, as these lesions were found even in obese horses or those not having any other body lesions. The statistically nonsignificantly higher (P>0.05) prevalence of the hip wounds and scars in the working horses during the winter compared to summer, confirm identical causality in both seasons; during the summer part of the animals were housed outside the barns, where they could find softer surfaces on the ground to rest.

Table 3 presents the differences between the horse categories assessed for the prevalence of welfare problem indicators in the frame of freedom from pain, injury or disease.

According to recent studies, the good quality of the hair coat could be a valuable indicator of good welfare. In the present study, the prevalence of normal hair coat was significantly lower in the working horses (P < 0.05) (**Table 3**), compared to stallions, broodmares and young horses during the warm season.

The injuries, on both the horse's body and legs, were more frequent in working horses than in those used for reproduction (**Table 3**). However, the risks for specific welfare problems are different, according to the way the horse is used. Among all the breeding horses, the highest exposure to the risk of injury was in the category of breeding stallions, because of their fights in the case of accidentally untying in the stables [15].

The lip corner lesions produced by the use of the bit and the injuries on the harness contact points were considered "marker" lesions for the improper use of the horses, their prevalence being significantly higher (P < 0.05) in this study within the working horses' group than in the animals used for breeding (**Table 3**). Some risk factors contributing to the development of the lesions at harness contact points are the thin body condition by losing the protection of the subcutaneous fat layer, the omission of brushing out the dirt from the horses' hair coat before harnessing and the usage of improper harnesses. Even though the working horses in

Indicators	% in each season and category								P
	WHw	WHs	BSw	BSs	BMw	BMs	RSw	RSs	
V. FREEDOM FROM FEAR AND DISTRESS									
General attitude									
Apathy	2.34	1.78	3.23	3.03	0	0	0	0	P>0.05
Response of the horse in the human approach test									
Aggressiveness	1.74	2.38	0	0	5.11	4.11	0	0	P>0.05
Fear/avoidance	22.23	20.84	22.58	25.76	22.62	25.34	0	0	P>0.05
Response of the horse in the human walk besides test									
Aggressiveness	4.68	5.37	0	0	5.84	6.16	0	0	P>0.05
Fear/avoidance	45.03^{a}	38.69^{ab}	30.65^{ab}	31.81^{ab}	35.77^{ab}	38.36^{ab}	0^{b}	0^{b}	P<0.05
Response of the horse in the human physical contact test									
Aggressiveness	5.26	8.34	0	0	8.76	8.22	0	0	P>0.05
Fear/avoidance	51.46^{a}	51.78^{a}	38.71^{ab}	43.94^{ab}	55.47^{a}	54.8^{a}	0^{b}	0^{b}	P<0.01

Table 5. The prevalence of welfare problems identified in the horses in different keeping conditions and seasons, within the investigation of the freedom from fear and distress. WHw = working horses, in winter; WHs = working horses, in summer; BSw = breeding stallions, in winter; BSw = breeding stallions, in summer; BMw = broodmares and young horses, in winter; BMs = broodmares and young horses, in summer; RSw = reproduction stallions, in winter; RSs = reproduction stallions, in summer; ns = difference not statistically significant (P > 0.05); P = significance of difference between categories and seasons; abc Values in a row with no common superscript are significantly different (P < 0.05).

developing countries present much more frequently all types of lesions comparing with the Romanian horses, these problems could be reduced further by the involvement of the horse owners in adopting better practices when working with the animals.

All the leg and feet problems showed higher prevalence in the working horses than the breeding animals, the differences being significant in several cases (**Table 3**). Besides the risks that are inherent for using the horses for work, part of these problems (such as the improper length and shape of the hooves) were produced by disregarding the good horse management practices and neglect, these problems being found also in the breeding horses (**Table 3**).

Understanding the importance of proper care for the hooves of the horses, even when the animals are not used for work, is most important as the occurrence of the disorders at this level can affect the quality of the gait, producing permanent abnormalities [16].

The respiratory disorders of the working horses were more frequent during the winter compared to the summer and were observed significantly more often in this category than in the other groups (**Table 3**). The influence of the air quality and microclimate conditions could not be overlooked, at least for the etiology of such diseases as recurrent airway obstruction or pulmonary emphysema (heaves) [17].

The prevalence of the situations when the assessed horses were restricted from displaying normal behaviors is presented in **Table 4**. In keeping conditions similar to the natural ones, both the access to social company of the conspecifics and to free exercise were ensured simultaneously. In this study, even if the majority of the working horses did not have the company of other horses, yet more than half of them were in the company of other mammals, probably the only advantage of mixed housing of several farmed species. The lack of possibilities to fulfill the need for free exercise in breeding stallions and many of the working horses was the aspect of highest concern (**Table 4**). It was observed, also, that those involved in taking care

for the horses (owners and employed caretakers) were not aware of the fact that neither exercise during draft work nor training cannot replace the free exercise for the animals.

The general apathetic attitude, the lack of response to the environmental stimuli can indicate severe disease or extreme physical or mental fatigue [18–19]. This behavior was noted only in a small percentage of the horses, without significant differences between the categories or the assessment season (**Table 5**). The frequency of the indifference or friendly response to the assessor is not presented here, even if overt indifference, along with depressed mental state, can indicate extremely severe welfare problems, such as learned helplessness [20].

It was observed that for each behavioral response type (aggressiveness, fear, indifference or friendliness), even if statistically significant differences were found between the assessed horse categories, the season and repetition of the assessment, in the majority of the cases on the same animals, did not have significant influence on the variability of the response in the behavioral tests. This suggested the existence of a temperamental trait, named by the researchers "reactivity to humans," stable in time and across situations. The study of these behavioral responses, in the light of present knowledge regarding human-animal relation, allowed the estimate of the behavior of humans during their interactions with the studied horses, as these animals respond to human proximity according the way they perceived previously the interspecific interaction. Knowing this aspect and manipulating horses appropriately by all those involved in keeping and caring for these animals is important, not only for contributing to the animals' positive mental experiences but also for human safety during work [21–22].

Figure 1 presents the distribution of the assessed horse population depending on the individual welfare scores and **Figure 2** shows the prevalence of the animals after their classification into the qualitative welfare categories, based on the obtained individual scores.

The working animals had the lowest individual scores, especially during the winter. About a quarter (25.6%) of these horses were classified as having acceptable welfare when assessed in summer, even though their number was lower in winter (18.13%) (**Figure 2**). The most concerning situation was that of the horses being in the unacceptable welfare category, as the improvement of their life quality represented an emergency.

The overall situation of the breeding stallions (**Figure 3**) was more problematic than that of the broodmares and young horses, the breeding stallions having higher prevalence of minimal welfare situations (17.75% in winter and 9.09% in summer), when compared to the broodmares and young horses (4.38% in winter and only 1.37% in summer).

Considering that the individual welfare scores are composed by the partial scores assigned for all the indicators assessing the five freedoms of animal welfare, the prevalence of the higher scores showed an overall better situation, regarding all the aspects of horse welfare.

Maximal scores (between 46 and 50) were recorded only for the broodmares and young horses (23.35% in winter and 31.1% in summer) (**Figure 1**) and for more than 90% of the reproduction

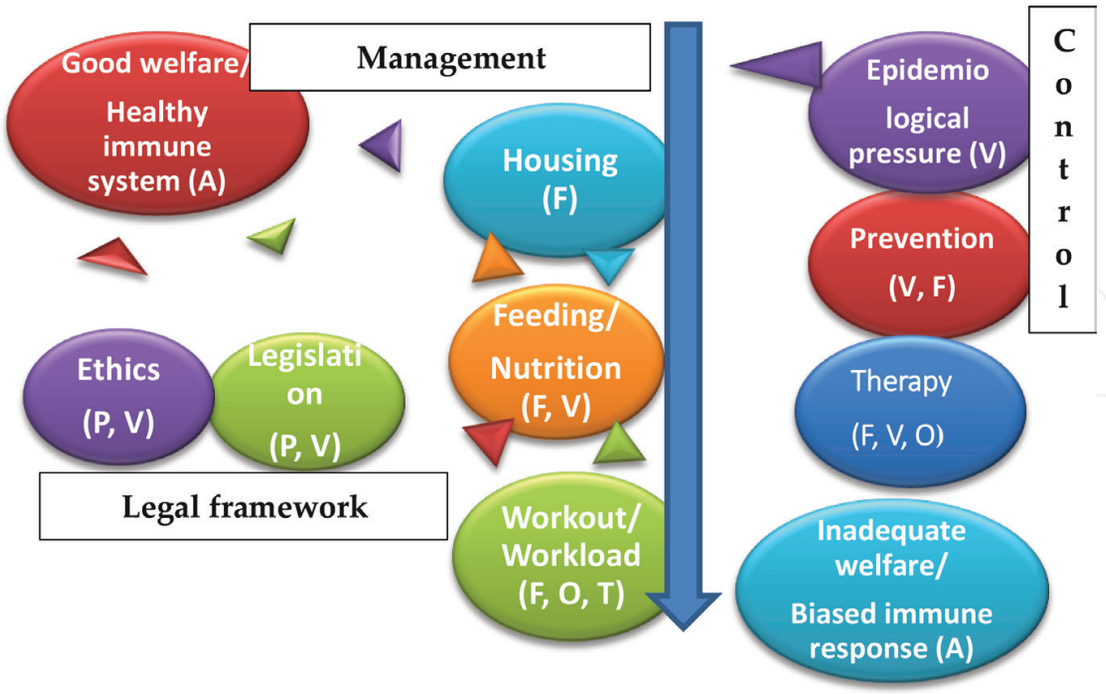

Figure 1. Some of the factors/interventions influencing the progression from good to poor welfare of the animal (A-animal, P-political decisions, V-veterinarian intervention, F-farmer conduct, O-owner conduct).

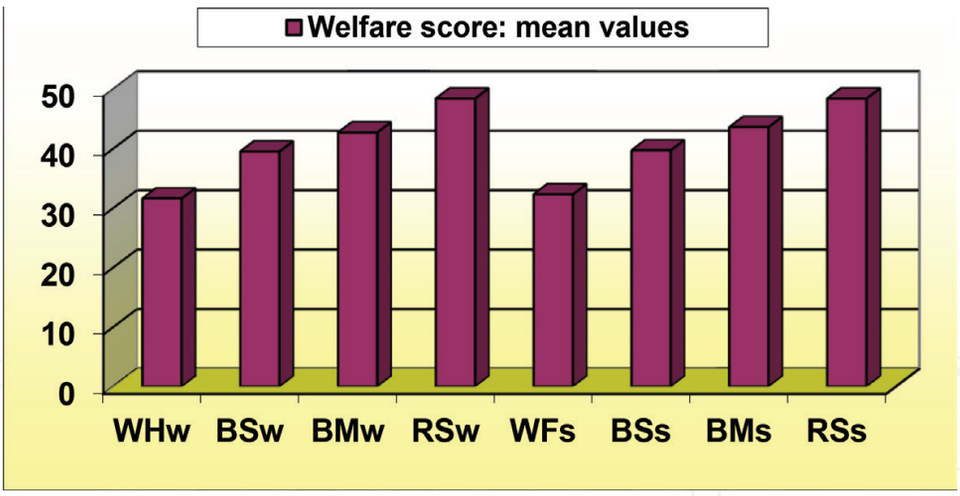

Figure 2. Mean values for individual welfare scores in horses. WHw = working horses, in winter; WHs = working horses, in summer; BSw = breeding stallions, in winter; BSw = breeding stallions, in summer; BMw = broodmares and young horses, in winter; BMs = broodmares and young horses, in summer; RSw = reproduction stallions, in winter; RSs = reproduction stallions, in summer.

stallions, in both seasons (**Figure 2**). For further increase, the prevalence of good welfare scores is important to provide good housing and management conditions to horses, but also the possibility to meet species-specific needs [23].

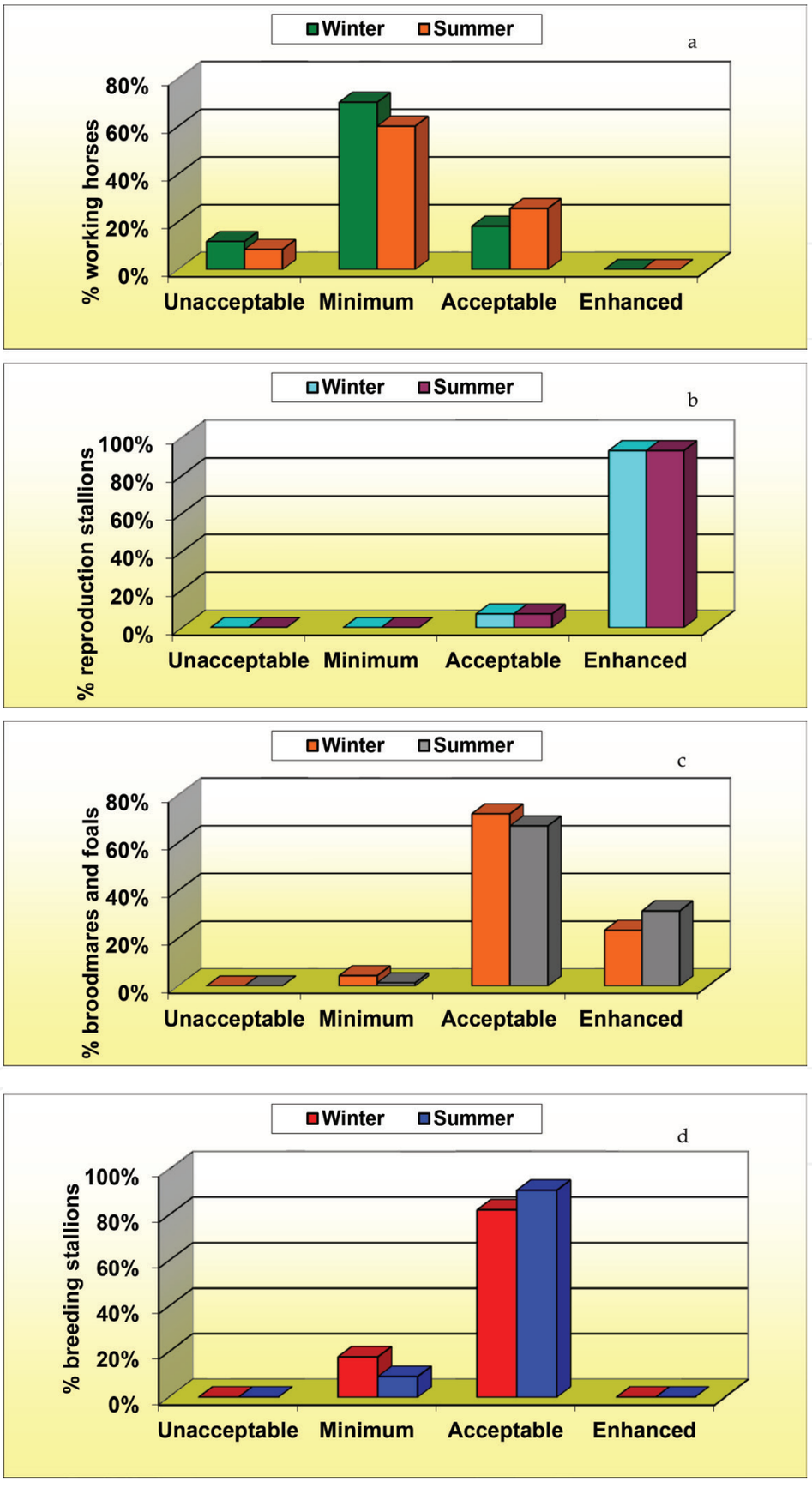

Figure 3. The distribution of the individual welfare scores according to the four qualitative classes, season and horse categories (a) working horses, (b) reproduction stallions, (c) broodmares and foals, (d) breeding stallions.

3. Assessment of immunological parameters involved in resistance to microbial diseases in horses

3.1. Methods

In order to assess the immune status and the defense potential against infectious diseases of the animals in experimental groups, differential white blood cell counts, total serum immunoglobulins and circulating immune complexes (CIC), the bactericidal capacity of their serum as well as *in vitro* phagocytic activity and *in vitro* blast transformation ability of leukocytes were tested [24–25]. Furthermore, the N/L ratios were calculated as indicators of stress.

The differential white blood cell count and the N:L ratio were performed for all of the studied horses; for the other tests in both evaluations (in the winter and in summer) 15 samples were randomly selected from each horse category, and 5 from the group of reproduction stallions, respectively.

3.2. Results and discussion

The mean results for the N:L ratio (**Table 6**) varied, showing significant differences in several cases, depending on the category of the tested horses and on the season.

The higher values of the N:L ratio in the majority of the working horses might indicate their constant exposure to acute bouts of stress and, most probably, also to chronic stress. In similar manner, although of lower intensity compared to working horses, the N:L ratio suggested significantly higher stress levels in the breeding stallions as opposed to the reproduction stallions. The fact that the improper housing and management, along with deprivation of the basic needs can lead to stress that can become chronic in animals, with all the negative consequences, has already been proven. In the opinion of some authors, a N:L ratio around 3 indicates an impairment of the health status which cannot be restored without substantial changes in the working schedule or management system.

The immunoglobulins had low mean values (**Table 7**) in all horse categories, in both seasons.

From the point of view of health status and disease resistance of an animal, the decrease of the adaptive immune system's functionality can be disastrous, exposing the host to a variety of opportunistic infections. The persistent lowering in the antibody production may occur in chronic stress (because of the endogenous production and release of corticoids) or if long-term/high doses of exogenous corticosteroids are given; also in immunodeficiency syndromes, incurable diseases or severe malnutrition. The variations in the total serum immunoglobulin levels which were observed in horses with no clinical illness signs proved the usefulness of this indicator in detecting subclinical disorders [27–28]. Nonetheless, it has to be considered that the serum concentration of total immunoglobulins depended, besides the antibody production rate, on their complexation rate with obtainment of circulating immune complexes and the clearance of the latter. For this reason, the results need to be corroborated for these two parameters.

The values for the concentration of circulating immune complexes (CIC) were relatively similar in the breeding horses and higher in the working animals, but did not exceed the normal

Parameter	Normal values*	WHw	WHs	BSw	BSs	BMw	BMs	RSw	RSs
WBC	5.6 – 12.1	10.71^{ab}	11.03^{a}	9.09^{ab}	8.49^{ab}	8.63^{ab}	9.05^{ab}	8.31^{b}	8.43^{b}
Neutrophils	2.9 – 8.5	7.42^{ab}	7.83^{a}	5.82^{ab}	5.26^{ab}	4.97^{ab}	5.22^{ab}	4.81^{b}	4.64^{b}
Lymphocyte	1.2 – 5.1	2.39^{ab}	2.30^{a}	2.77^{ab}	2.63^{ab}	2.76^{ab}	2.74^{ab}	3.0^{b}	3.1^{b}
Monocytes	0 – 0.7	0.6	0.6	0.3	0.1	0.3	0.2	0.1	0.1
Eosinophils	0 – 0.8	0.3	0.2	0.2	0.3	0.6	0.6	0.4	0.4
Bazophils	0 – 0.3	0	0.1	0	0.2	0	0.1	0	0.1
N:L ratio	** 0.8 – 2.8	3.1^{a}	3.4^{a}	2.1^{b}	2.0^{b}	1.8^{bc}	1.9^{bc}	1.6^{bc}	1.5^{c}

Table 6. Mean values of the white blood cell counts (×109/L), including the N:L ratio, in the studied horses [25–26]. WHw = working horses, in winter; WHs = working horses, in summer; BSw = breeding stallions, in winter; BSw = breeding stallions, in summer; BMw = broodmares and young horses, in winter; BMs = broodmares and young horses, in summer; RSw = reproduction stallions, in winter; RSs = reproduction stallions, in summer; abc Values in a row with no common superscript are significantly different ($P < 0.05$).

limits in none of the horse categories. The results in the winter testing were higher than in summer, excepting the broodmares and young horses. This tendency could be the outcome of prolonged housing during the winter, in the presence of higher antigenic stimulation, compared to the periods of the year when the horses spent more time outdoors. Even in the breeding stallions, that were permanently housed, the lower ventilation rates during the winter might have increased the antigenic load in the barns' microclimate. Higher CIC values in the working horses might be due to the housing of the horses, usually along with other animal species. This housing type could lead to increased antigenic stimulation, even if part of the microorganisms was specific for a single animal species. In the same time, the management of barn cleaning, probably better in the organized conditions of a farming system, could strongly influence the microbial load and presence of abiotic antigens in the barns.

The phagocytic index was the highest for the first reading at 45 min, especially in the working horses evaluated during winter and in the broodmares and young horses during the summer. In all horse categories, the phagocytic activity decreased subsequently. This tendency was not observed in breeding stallions, with a more efficient overall phagocytic activity [29]. Attempts to monitor the *in vitro* efficacy of an alcoholic milk thistle (*Silybum marianum*) extract as an phagocytosis enhancing agent did not deliver a positive outcome (**Table 8**) (**Figure 4**).

Parameter	WHw	WHs	BSw	BSs	BMw	BMs	RSw	RSs
Sample size	15	15	15	15	15	15	5	5
Minimum	0.264	0.218	0.247	0.236	0.242	0.228	0.258	0.258
Maximum	0.468	0.378	0.374	0.314	0.345	0.342	0.338	0.338
Mean	0.342^{a}	0.296^{b}	0.314^{ab}	0.283^{b}	0.292^{b}	0.287^{b}	0.292^{ab}	0.297^{ab}
Median	0.327	0.299	0.314	0.286	0.287	0.284	0.272	0.301
Standard deviation	0.059	0.047	0.038	0.022	0.031	0.034	0.034	0.033
Standard error of the mean	0.015	0.012	0.009	0.006	0.008	0.009	0.015	0.015

Table 7. Descriptive statistical parameters of the total serum immunoglobulin concentrations (expressed in optical density units ODU) in the studied horses. WHw = working horses, in winter; WHs = working horses, in summer; BSw = breeding stallions, in winter; BSw = breeding stallions, in summer; BMw = broodmares and young horses, in winter; BMs = broodmares and young horses, in summer; RSw = reproduction stallions, in winter; RSs = reproduction stallions, in summer; abc Values in a row with no common superscript are significantly different ($P < 0.05$).

Variant		CMi WHw	CMv WHs	AMPi BSw	AMPv BSs	IMi BMw	IMv BMs	APi RSw	APv RSs
Control	ln45-ln0/45	0,006	0,011	0,001	-0,003	0,014	0,011	0,007	0,006
	ln60-ln45/15	0,004	-0,001	-0,004	-0,002	0,0001	0,001	0,003	0,001
Alcohol	ln45-ln0/45	0,002	0,008	-0,001	0,001	0,031	0,033	0,011	0,001
	ln60-ln45/15	0,003	0,0001	-0,002	-0,005	0,005	0,006	0,002	0,003
Sylibum marianum	ln45-ln0/45	0,0003	0,003	-0,001	-0,001	-0,001	0,001	0,001	0,001
	ln60-ln45/15	0,002	0,002	0,001	0,007	0,002	0,004	0,002	0,004

Table 8. Mean values of the phagocytic index in the studied horses. WHw = working horses, in winter; WHs = working horses, in summer; BSw = breeding stallions, in winter; BSw = breeding stallions, in summer; BMw = broodmares and young horses, in winter; BMs = broodmares and young horses, in summer; RSw = reproduction stallions, in winter; RSs = reproduction stallions, in summer.

The *in vitro* blast transformation of leukocytes aimed at quantifying the functional ability of the cells as a measure of potential antimicrobial reactivity, but also at investigating the effects of several mitogens and plant extracts on the immune response to select compound able to alleviate the stress effects on the immune system. For that, 12 variants were performed for each tested sample. The control culture (spontaneous blast transformation, no addition) had the lowest growth index in the broodmares and young horses, while the highest values were recorded for the breeding stallions, during both seasons. The oat (*Avena sativa*) extract had most frequently an inhibitory effect on growth, while the shepherd's purse (*Capsella bursa-pastoris*) extract showed stimulating effects, especially during winter. The enhanced stimulating effects of different plant extracts were associated with keeping conditions closer to the natural ones.

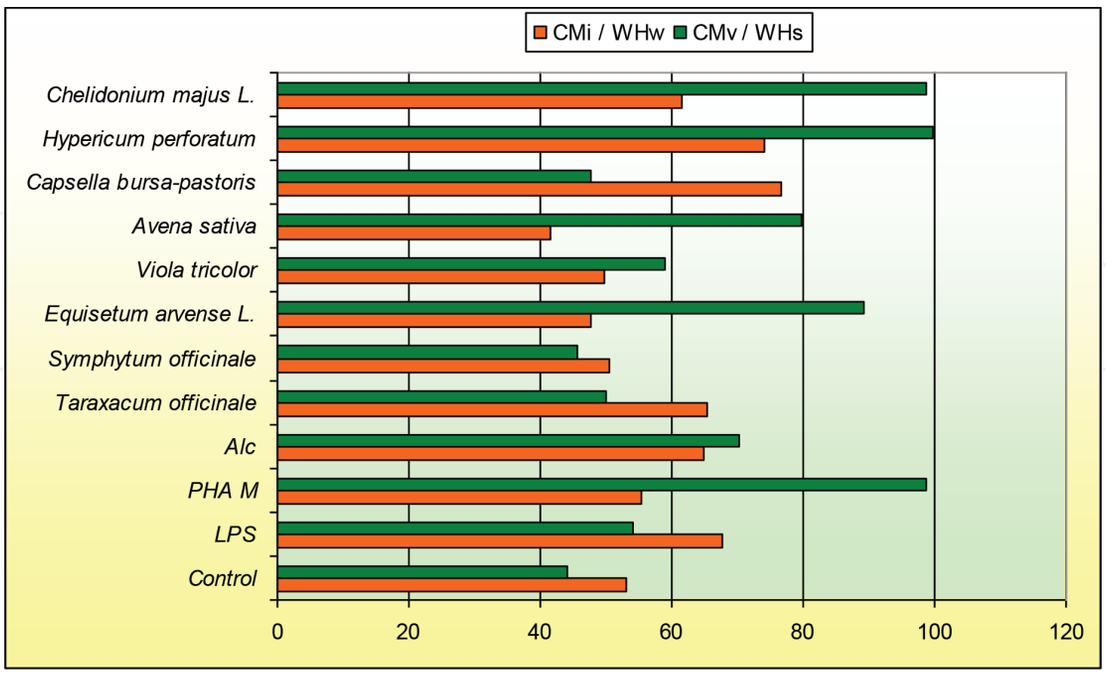

Figure 4. Mean values of the blastic transformation indices (%) in the working horses during summer and winter (WHw = working horses, in winter; WHs = working horses, in summer; Alc = alcohol; PHA M = phytohemagglutinin M; LPS = lipopolysaccharide from *E. coli* cell wall).

The bactericidal capacity of the serum in the studied horses was superior (both as efficiency and as duration) against the gram positive bacteria (*Streptococcus spp.*), compared to that against the gram negatives (*Escherichia coli*). Even if in the stallions (breeding and reproduction), the mean values of the results were higher and the bactericidal activity was observed more in the successive dilutions, versus the other horse categories, yet this nonspecific immune defense mechanism was not influenced by the keeping conditions and management of the animals.

4. Investigation of the relationships between the welfare degree and immune reactivity in the assessed horses

To investigate the relationship between well-being and the immune status of the horses, the data were analyzed using the SPSS statistical program (version 17). Spearman rank (rs) correlation coefficients were calculated for the study of the relationship between the variables, since the obtained data did not show a normal distribution. Correlations were considered significant at the significance threshold $P < 0.05$, distinctly significant at the threshold $P < 0.01$ and very significant at $P < 0.001$.

Category	N/L ratio	Total Ig	CIC	Blast transformation
Working horses, in winter				
Individual welfare score	-0,41**	-0,54*	-0,48**	-0,27*
Stimulation index PHA M	-0,31*		0,37*	
Total immune globulins	-0,38*			
Working horses, in summer				
Individual welfare score	-0,46**			-0,21*
Stimulation index		-0,26*		
Breeding stallions, in winter				
Individual welfare score	-0,38*	0,64*	0,36*	0,2*
Individual welfare score, summer	-0,22*			
CIC breeding stallions, winter	0,61*	0,43*		
Breeding stallions, in summer				
Individual welfare score	-0,39*			0,32*
Individual welfare score, winter	-0,21*			
CIC breeding stallions	0,22*	0,38*		
Total immune globulins	-0,38**			
Broodmares,young horses, in winter				
Individual welfare score	-0,27**	0,21*		
Total immune globulins	-0.32**			
Stimulation index control culture	-0,28*			
CIC		-0,52**		
Broodmares, young horses, summer				
Individual welfare score	-0,33**		0,27*	0,32*
Individual welfare score, winter	-0,23**			
CIC		0,64**		
Stimulation index control culture		0,72**		
Stimulation index *H.perforatum*		0,36**		

Table 9. Significant correlations found between individual welfare scores and several immunological indicators in the studied horses.

Of all the indicators, the N:L ratio correlated most frequently with the welfare score of the assessed horses. The increase of this ratio showed the acute stress response in the first place, but through negative correlations, it showed also the presence of chronic stress in horses.

The increased concentration of the immunoglobulins was associated with those keeping conditions where the antigenic pressure was high, indicating a stronger adaptive response as the animals' welfare degree was higher. In those horses kept in near-natural conditions, the concentration of CIC indicated the functionality of the normal processes of antibody synthesis and clearance by immune complexation. Interpreting the correlations between CIC concentrations and the individual welfare score needed the additional consideration of total serum immunoglobulins, to differentiate between the chronic disease status and lack of antigenic stimulation. The increase of phagocytic indices in the horses with low individual welfare scores could indicate the participation of hypoxia producing chronic diseases in the enhancement of phagocytosis. The few correlations obtained between the indices of blast transformation and the individual welfare scores showed the concomitant variation of these two parameters. In the case of the working horses in winter, a drop in the immune reactivity was possible on the background of a general hyporesponsiveness, involving the chronic stress caused by the environment.

According to the results, the bactericidal capacity of the serum proved to be more intense in those situations where the antigenic pressure was increased, and its efficiency was improved as the animals had a higher degree of welfare (**Table 9**).

5. Conclusions

This study, including various horse categories from Romanian raising systems indicated that there was a variation of welfare drawbacks depending on the workout category. In working horses, there was the highest prevalence of welfare problems, such as the lack of constant access to drinking water, the lack of comfortable resting surface, the harness-caused lesions, the health problems of the legs, the inadequacy of shoeing practices, the lack of conspecific company and lack of access to free exercise. The major welfare problems of the reproduction horses and breeding stallions were the lack of permanent access to drinking water and the lack of free exercise possibility, respectively. The human-horse relationship represented a welfare problem, whose suitability was difficult to assess in all of the studied horse categories.

Neutrophil:lymphocyte ratios, total serum immunoglobulins and the circulating immune complexes' levels helped identifying situations which impose an intense adaptive response and immune defense, triggered either by chronic or acute stress or an increased antigenic pressure in the habitat.

The correlated use of the welfare assessment by direct indicators with the investigation of the immune status proved to be a powerful tool in the interdisciplinary research on ways the horses cope with the demands imposed by different management conditions. The dimensions

of this approach have not been exhausted by far in this study, which is merely a first step taken in our country to a better understanding of how these animals could be simultaneously used and protected.

Conflict of interest

None of the authors have any existing or possible conflict of interest, including financial, personal or any other relationship which could influence their scientific work.

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